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MINIREVIEW

Base Excision Repair, a Pathway Regulated by Posttranslational Modifications

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Base excision repair (BER) is an essential DNA repair pathway involved in the maintenance of genome stability and thus in the prevention of human diseases, such as premature aging, neurodegenerative diseases, and cancer. Protein posttranslational modifications (PTMs), including acetylation, methylation, phosphorylation, SUMOylation, and ubiquitylation, have emerged as important contributors in controlling cellular BER protein levels, enzymatic activities, protein-protein interactions, and protein cellular localization. These PTMs therefore play key roles in regulating the BER pathway and are consequently crucial for coordinating an efficient cellular DNA damage response. In this review, we summarize the presently available data on characterized PTMs of key BER proteins, the functional consequences of these modifications at the protein level, and also the impact on BER *in vitro* and *in vivo*.

It has been estimated that every day, each human cell generates >10,000 DNA base lesions as a consequence of the instability of DNA, caused by hydrolysis, cellular oxidative metabolism, and environmental factors, including ionizing radiation (IR) (1). Such sites of DNA base damage include base loss (apurinic/apyrimidinic [AP] sites), DNA base modifications (e.g., base alkylation and oxidation), and DNA single-strand breaks (SSBs), which are a major threat to the integrity of the human genome. In the 1970s, Tomas Lindahl (corecipient of the 2015 Nobel Prize in chemistry) was the first to identify a DNA *N*-glycosylase, namely, uracil DNA *N*-glycosylase (UNG), that excises uracil residues from DNA (2). Lindahl recognized that following UNG activity, an endonuclease, a DNA polymerase (Pol), and a DNA ligase (Lig) would be required to complete the "excision-repair" process, and this formed the starting point for the identification of the base excision repair (BER) pathway. Since then, most of the major enzymes involved in BER have been identified and characterized in terms of their roles and enzymatic activities.

BER is a coordinated process (Fig. 1) and in humans is initiated by 1 of 11 damage-specific DNA glycosylases. These enzymes display substrate specificity for particular types of damaged DNA bases and employ a "base-flipping" mechanism to excise these DNA lesions (3, 4). There are two different types of DNA glycosylases, monofunctional glycosylases (DNA glycosylase activity only) and bifunctional glycosylases (DNA glycosylase plus DNA strand cleavage activities). Monofunctional DNA glycosylases sever the *N*-glycosidic bond between the damaged base and the phosphodiester DNA backbone, creating an AP site. The AP site is recognized by AP endonuclease 1 (APE1), which cleaves the DNA backbone, resulting in the formation of a one nucleotide gap flanked by 3′-hydroxyl and 5′-deoxyribosephosphate (5′-dRP) ends (5, 6). Conversely, bifunctional DNA glycosylases, in addition to performing base damage removal, incise the DNA backbone to create a single-nucleotide gap flanked by either a 5′ phosphate and a 3′-α,β-unsaturated aldehyde (termed β-elimination) or 5′-phosphate and 3′-phosphate residues (termed β,δ-elimination). The DNA glycosylases known to catalyze β-elimination are 8-oxoguanine (8-oxoG) DNA glycosylase (OGG1) and the endonuclease III homologue (NTH1). Following their activity, the 3′-α,β-unsaturated aldehyde is excised by APE1 to generate a 3′-hydroxyl end, which is the same product as that derived from monofunctional DNA glycosylase plus APE1 action. However, due to the abundance of cellular APE1, combined with the low efficiency of the β-elimination activities shown by OGG1 and NTH1, APE1 can bypass this step and cleave the AP site itself (7). The enzymes catalyzing β,δ-elimination are endonuclease VIII-like proteins 1 to 3 (NEIL1 to -3), and the 3′-phosphate generated by their activity is excised by polynucleotide kinase phosphatase (PNKP) (8). Consequently, APE1-dependent and APE1-independent (PNKP-dependent) pathways of BER have been identified (Fig. 1, central and left pathways, respectively). Nevertheless, the common end product of the combination of DNA glycosylase activity and APE1 or PNKP activity is the generation of a single-nucleotide gap containing a 3′-hydroxyl end which is a substrate for a DNA polymerase. Indeed, the major DNA polymerase employed in BER is DNA polymerase β (Pol β). Pol β acts by removing 5′-dRP moieties remaining from the activity of DNA glycosylase plus APE1 and simultaneously inserts the correct undamaged nucleotide into the repair gap (9, 10). A complex consisting of DNA ligase IIIα (Lig III) and X-ray cross-complementing protein 1 (XRCC1) then seals the remaining nick in the DNA backbone (11, 12). This completes the BER process through the repair and replacement of a single damaged DNA base. This is the predominant mode of BER (~80% of all events) and is commonly referred to as short-patch BER (13). In some circumstances (e.g., when the 5′-dRP residue is oxidized or reduced and resistant to excision by Pol β), following addition of the first nucleotide by Pol β to initiate repair, a polymerase switch to the replicative enzyme DNA polymerase δ or ε (Pol δ/ε) occurs (14). Pol δ/ε acts by adding several (typically 2 to 8) nucleotides into the single-nucleotide gap, generating a 5′-DNA flap structure, which is excised by flap

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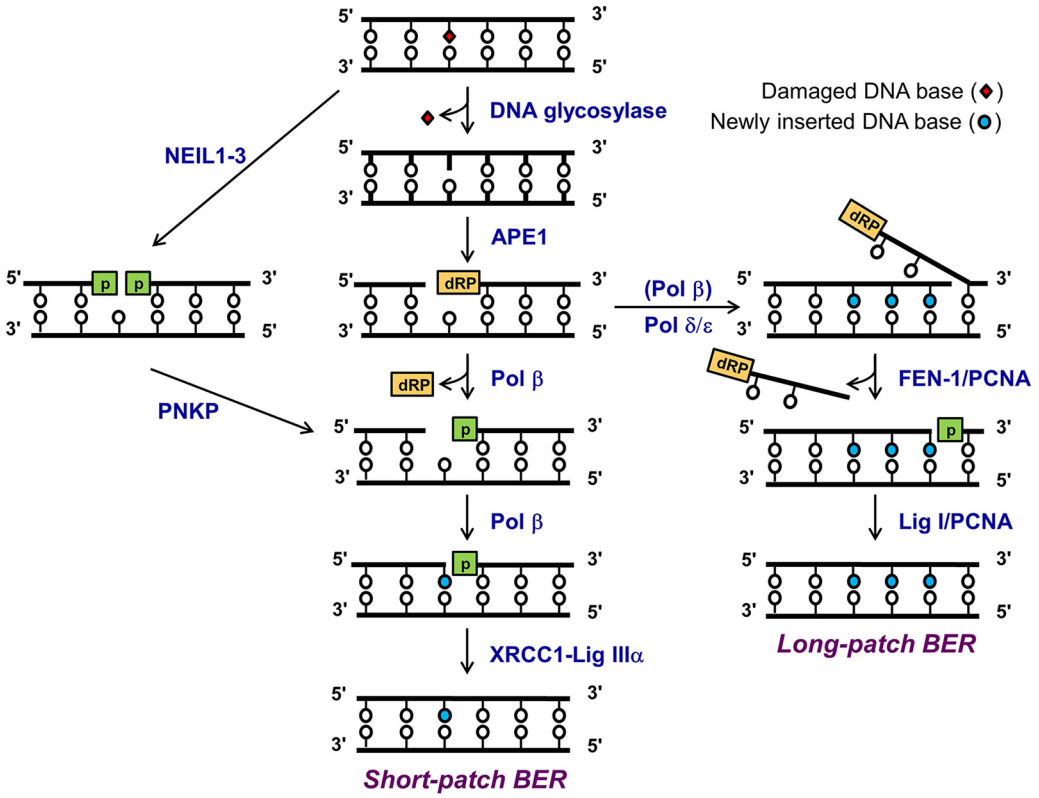

FIG 1 Repair of DNA base lesions by the human BER pathway. Damage-specific DNA glycosylases (such as OGG1, NTH1, and UNG) excise the damaged base by cleavage of the *N*-glycosidic bond, leaving an AP site. This is incised by APE1 to create a single-strand break containing a 5′-dRP moiety, which is removed by the dRP lyase activity of Pol β that simultaneously fills the gap with a new nucleotide (central branch). In contrast, BER initiated by the NEIL DNA glycosylases (NEIL1, NEIL2, and NEIL3) possess β,δ-elimination activity which creates a single-nucleotide gap containing 3′- and 5′-phosphate ends. The 3′-phosphate is removed by PNKP before nucleotide insertion by Pol β (left branch) occurs. Finally, the remaining nick in the phosphodiester backbone is sealed by a Lig III-XRCC1 complex to complete short-patch BER. If the 5′-dRP moiety is resistant to Pol β activity, then a polymerase switch to Pol δ/ε occurs. These DNA polymerases add 2 to 8 more nucleotides into the single-nucleotide gap, creating a 5′-flap structure which is recognized and excised by the endonuclease activity of FEN-1, in a PCNA-dependent manner (right branch). The remaining nick in the DNA backbone is then repaired by DNA ligase I, also in association with PCNA, to complete long-patch BER.

endonuclease-1 (FEN-1) in a proliferating cell nuclear antigen (PCNA)-dependent process. Finally, DNA ligase I (Lig I) in concert with PCNA seals the remaining nick in the DNA backbone, completing the long-patch BER pathway (Fig. 1, right pathway) (14, 15).

POSTTRANSLATIONAL MODIFICATION OF HUMAN BER PROTEINS

Since the discovery of the BER pathway, and particularly in the last couple of decades, it has become evident that BER as a whole, but specifically the proteins involved in the repair process, is controlled and regulated by site-specific posttranslational modifications (PTMs). These PTMs involve acetylation, methylation, phosphorylation, SUMOylation, and ubiquitylation catalyzed by acetyltransferases, methyltransferases, protein kinases, SUMO ligases, and E3 ubiquitin ligases, respectively. These PTMs can have widely diverse effects on protein function and may alter enzymatic activity, cellular localization, protein-protein interactions, and/or cellular protein levels. There is also accumulating evidence of significant cross talk between PTMs in regulating BER protein functions. These mechanisms can subsequently control the BER response relative to the changing DNA damage environment and can prevent the accumulation of toxic and mutagenic DNA bases and BER intermediates associated with increased genome instability. In the next section, we summarize the known characterized PTMs of human BER proteins catalyzed by specific enzymes and highlight the significance of these modifications for protein function and the relevance for cellular BER activity.

DNA GLYCOSYLASES

UNG. Uracil DNA glycosylase (UNG) is involved in the repair of uracil and its derivatives (e.g., 5-hydroxyuracil), which may have been misincorporated into newly synthesized DNA opposite adenine residues or produced following the deamination of cytosine. UNG2 is the nuclear form of the protein, whereas UNG1 is mitochondrial. UNG2 is sequentially phosphorylated *in vivo* by cyclin-dependent kinases (Cdk) on serine 23, threonine 60, and serine 64 (S23, T60, and S64, respectively) during the S phase of the cell cycle (16). The initial phosphorylation on S23 in the late G_1/early S phase promotes association of UNG2 with replication protein A (RPA) and likely stimulates the enzyme to excise uracil residues in replicating DNA. Subsequent phosphorylation of T60 and S64 reduces UNG2 binding to RPA and codes for the glycosylase to be degraded later in the G_2 phase of the cell cycle. Additional UNG2 residues have been identified as being phosphorylated *in vivo*, specifically in response to UV irradiation. Phosphorylation of threo-

nine 6 and threonine 126 (T6 and T126, respectively) was shown to result in increased DNA glycosylase activity *in vitro*, and this can be reversed by the p53-induced phosphatase PPM1D/Wip1, acting specifically at T6, which is sufficient to reduce UNG2 activity (17).

UNG2 has also been suggested to be susceptible to SUMOylation and ubiquitylation. When SUMO-1 was overexpressed in HepG2 liver cancer cells, increased protein levels of UNG2 were detected, suggesting that SUMOylation protects the enzyme from degradation (18). However, direct evidence of UDG SUMOylation, and of the proposed inhibition of ubiquitylation-dependent degradation, was not provided. Ubiquitylation-dependent degradation of UNG2 has, however, been discovered during the S phase of the cell cycle, when phosphorylation has also been shown to occur. Indeed, there was an abrogation of UNG2 degradation in cells treated with the Cdk inhibitor roscovitine (19). This suggests that phosphorylation either induces a structural change in UNG2, which may be a prerequisite for ubiquitylation, or perhaps promotes nuclear export of the protein. The scaffold proteins cullin 1 (Cul1) and cullin 4 (Cul4) (in association with damage-specific DNA binding protein 1 [DDB1]), which can participate in multiple E3 ubiquitin ligase complexes, have been identified as enzymes that execute UNG2 ubiquitylation and subsequent proteasomal degradation *in vivo* (20, 21). Although UNG2 degradation was observed only following viral infection, a direct effect of Cdk-mediated UNG2 phosphorylation on stimulation of ubiquitylation-dependent degradation by Cul1/Cul4 complexes has not yet been demonstrated. Furthermore, the role of these ubiquitin ligases in controlling cellular UNG2 protein levels, particularly in the BER response to DNA damage, is currently unknown.

SMUG1. Single-strand-selective monofunctional uracil glycosylase 1 (SMUG1) is another enzyme that excises uracil residues, predominantly from U-G mismatches in nonreplicating chromatin arising from the deamination of cytosine. The only evidence of SMUG1 regulation by PTMs is through ubiquitylation. Similarly to UNG2, SMUG1 can be ubiquitylated *in vivo* by Cul1- and Cul4-based ubiquitin ligases, with the Cul4 complex likewise dependent on the presence of DDB1 (20, 21). The Vpr accessory protein of HIV-1 has been shown to interact with Cul4A within the DDB1-Cul4A complex and induces the degradation of both UNG2 and SMUG1, presumably via the ubiquitin proteasome pathway (UPP) (20–22). As mentioned above for UNG2, the relevance of Cul1/Cul4-based ubiquitin ligases in modulating SMUG1 protein levels required for cellular BER has not yet been studied.

MBD4. The methyl CpG binding domain protein 4 (MBD4) glycosylase excises uracil and thymine and their derivatives (e.g., 5-hydroxyuracil) that have been mispaired with guanine in CpG dinucleotides. The only evidence to date suggesting that MBD4 is regulated by PTMs is through phosphorylation. MDB4 was phosphorylated *in vitro* and *in vivo* by protein kinase C (PKC) on serines 165 and 262 (S165 and S262, respectively) (23). This PKC-dependent phosphorylation was shown to enhance the *in vitro* DNA glycosylase activity of MBD4 on methylated CpG oligonucleotides and subsequently promoted incision of methylated DNA following parathyroid hormone stimulation *in vivo*. This suggests a role for BER, mediated by MBD4 phosphorylation, in hormone-induced DNA demethylation and in subsequent transcriptional derepression. Additionally, MBD4 has recently been shown to directly interact *in vitro* and *in vivo* with the E3 ubiquitin ligase ubiquitin-like with PHD and RING finger domains 1 (UHRF1) and with the deubiquitylation enzyme ubiquitin-specific protease 7 (USP7) (24). It is therefore interesting to speculate that MBD4 may be also regulated by ubiquitylation.

TDG. Thymine DNA glycosylase (TDG) recognizes uracil, thymine, and 5-hydroxymethyluracil in mismatches with guanine in double-stranded DNA, as well as excising oxidative derivatives of 5-methylcytosine. TDG is acetylated *in vitro* and *in vivo* by CREB-binding protein and p300 (CBP/p300) at lysine residues 94, 95, and 98 (K94, K95, and K98, respectively), and this PTM was suggested to prevent recruitment of APE1, thus suppressing downstream DNA repair activities (25). Interestingly, TDG is also phosphorylated by PKC on serine residues 93, 96, and 99 (S93, S96, and S99, respectively) *in vitro*, and these phosphorylation sites are near those acetylated by CBP/p300. It has been proposed that, since acetylation prevents DNA repair, phosphorylation may actually promote repair by blocking adjacent lysine residues and preventing their acetylation (26).

The best-characterized PTM of TDG is SUMOylation. It was first proposed that modification of TDG on lysine 330 (K330) by SUMO-1 *in vitro* reduces the DNA binding affinity of the enzyme and thus stimulates dissociation from the products of base excision, AP sites (27). This was further supported by evidence indicating that *in vitro* SUMOylation of TDG induces a conformational change which promotes enzyme dissociation from the AP site (28) and that this also increases enzymatic turnover of TDG by overcoming product inhibition (29). Attachment of SUMO-1 or SUMO-2 to TDG *in vitro* has been shown to occur just as efficiently whether TDG is free or DNA bound, although SUMOylation was confirmed in reducing the binding affinity of TDG to DNA (30). In addition, SUMOylation of TDG on lysine 341 (K341) by SUMO-1 has been suggested to inhibit the enzyme's interaction with CBP *in vitro*. This would therefore prevent acetylation of TDG, which is thought to inhibit interaction of the enzyme with APE1. Thus, a role for SUMOylation in the enhancement of TDG-mediated DNA damage repair has been proposed (31).

TDG has recently been shown to be susceptible to ubiquitylation-dependent degradation via the UPP *in vivo*, specifically during the S phase. This process is promoted by the E3 ubiquitin ligase complex Cul4-DDB1-RBX1 in association with Cdt2 (cumulatively named "$CRL4^{Cdt2}$") and is dependent on the interaction of TDG with PCNA (32, 33). The site of ubiquitylation within TDG was not identified, and the influence of other PTMs (i.e., acetylation, phosphorylation, and SUMOylation) on the degradation process is currently unknown.

MYH. MutY glycosylase homologue (MYH) removes adenine residues that have been incorrectly paired with guanine bases or 8-oxoG oxidative DNA lesions. MYH was first shown to be present in a phosphorylated form in human cell extracts, and dephosphorylation reduced its DNA glycosylase activity (34). Defective MYH activity *in vitro* was discovered using colorectal cancer cell lines and was proposed to be as a consequence of reduced phosphorylation of MYH. Accordingly, MYH repair activity was increased following serine phosphorylation by PKC (35). More recent data have indicated that MYH is phosphorylated on serine 524 (S524) *in vivo*; however, this modified protein constituted only a small fraction of the total MYH in cells (36). Phosphorylation was proposed to alter binding affinity to damaged DNA *in vitro* through the creation of MYH mutants but may also regulate repair by altering its ability to bind to PCNA. However, the precise

role of MYH phosphorylation *in vivo* in the context of BER requires further investigation.

MYH has been reported to be ubiquitylated by Mcl-1 ubiquitin ligase E3/ARF binding protein 1 (Mule/ARF-BP1) *in vitro* and *in vivo* on at least one of five C-terminal lysine residues, between amino acids 475 and 535. The mutation of these lysine residues stabilized MYH by preventing its degradation; likewise, a small interfering RNA (siRNA)-mediated knockdown of Mule increased endogenous MYH levels (37). A ubiquitylation-deficient mutant of MYH also showed enhanced binding to chromatin, suggesting that ubiquitylation additionally influences subcellular localization and/or DNA binding.

OGG1. 8-OxoG DNA glycosylase (OGG1) is the major DNA glycosylase for repairing 8-oxoG and 2,6-diamino-4-oxo-5-formamidopyrimidine (FapyG) DNA lesions. OGG1-1a is the major nuclear isoform discussed here, although seven other isoforms are present in the mitochondria. Acetylated OGG1, catalyzed by the enzyme p300, has been proposed to be present in human cells at a level representing approximately 20% of the entire cellular OGG1 pool (38). The acetylated residues were mapped to lysines 338 and 341 (K338 and K341, respectively) and were proposed to increase base excision activity by reducing the affinity of OGG1 for its AP site product. OGG1 acetylation was increased *in vivo* following oxidative stress, suggesting a DNA damage-dependent activation of OGG1 activity. In contrast, a more recent though limited study suggested that OGG1 acetylation is actually reduced *in vivo* following oxidative DNA damage by hydrogen peroxide treatment (39), so further studies examining OGG1 acetylation are required.

OGG1 was discovered to be phosphorylated in a small number of studies. First, chromatin-associated OGG1 was shown to be phosphorylated on serine residues *in vivo*, and the activity was thought to be performed by PKC (40). In support of this, a phosphorylated form of OGG1 was identified using phosphoserine-specific antibodies in chromatin fractions isolated from human cells (41). A separate study showed that phosphorylation of OGG1 was stimulated by two protein kinases, c-Abl and cyclin-dependent kinase 4 (Cdk4), both *in vitro* and *in vivo* (42). Phosphorylation by the tyrosine kinase c-Abl was deduced to have no effect on base excision activity, whereas the serine/threonine kinase Cdk4 stimulated OGG1 activity. Despite this evidence, more-recent data on the role of OGG1 phosphorylation, and on the kinases catalyzing this PTM, have been lacking.

Modification of OGG1 by ubiquitylation *in vivo* has been documented in one instance. This was catalyzed by the C terminus of HSC70-interacting protein (CHIP) E3 ubiquitin ligase, although the process occurred exclusively under hyperthermic conditions (43). Under these specific conditions, the OGG1 protein was rendered inactive and was relocated to a detergent-resistant protein fraction and then subjected to ubiquitylation-dependent proteasomal degradation.

NTH1. Endonuclease III homologue (NTH1) is known to excise oxidized pyrimidines, such as thymine glycol and 5-hydroxycytosine within DNA; however, regulation of the human enzyme by PTMs has not yet been reported.

NEIL1 to -3. The three glycosylases endonuclease VIII-like protein 1 (NEIL1), NEIL2, and NEIL3 remove a range of oxidized DNA base damage from both single-stranded and double-stranded DNA. Since they are the most recently discovered enzymes in BER, identified just over a decade ago, their regulation via PTMs is still understudied. NEIL2 has shown to be acetylated on lysines 49 and 153 (K49 and K153, respectively) by the acetyltransferase p300 *in vitro*. Acetylation of K153 was revealed to have no impact on the *in vitro* activity of NEIL2, whereas acetylation of K49 inactivated NEIL2 by impeding both its base excision and AP lyase activities (44). This evidence suggests that acetylation may regulate NEIL2-mediated BER, although no subsequent studies have shown that NEIL2 acetylation occurs *in vivo* or that it is important for overall cellular BER activity. No direct evidence for PTM of NEIL1 or NEIL3 by specific enzymes has been reported.

MPG. Methylpurine DNA glycosylase (MPG) is a DNA glycosylase that recognizes and repairs most types of alkylated base damage. MPG has been shown to be acetylated *in vitro* by p300, a modification that was further stimulated by estrogen receptor alpha (ERα) (45). While ERα increased binding of MPG *in vitro* to alkylated base damage and improved base excision, this was independent of acetylation. It remains to be seen whether MPG is a direct substrate for p300-dependent acetylation *in vivo*. The DNA glycosylase activity of MPG was, however, shown to be directly enhanced by phosphorylation at serine 172 (S172) by the ataxia telangiectasia mutated (ATM) kinase (46). Phosphorylation of MPG *in vivo* was furthermore required for efficient DNA repair following treatment with the alkylating agent temozolomide. This PTM of MPG consequently increased cell viability and reduced apoptosis, suggesting that phosphorylation is an important event in the cellular response to alkylated DNA base damage.

END PROCESSORS

PARP-1. While not specifically an end processor, poly(ADP-ribose) polymerase 1 (PARP-1) is known to have a high affinity for DNA SSBs which are BER intermediates. PARP-1 therefore plays an active role in the BER process (47), mediated through its ability to catalyze the addition of poly(ADP-ribose) (PAR) polymers to itself and other proteins. The development and use of PARP inhibitors for blocking BER and SSB repair, particularly in the killing of breast cancer 1 gene (BRCA1)-deficient tumors, have grown in the last decade, and therefore there is significant interest in PARP-1 regulation. Indeed, PARP-1 has been demonstrated to be subject to a number of PTMs. It has been shown that PARP-1 is acetylated *in vitro* and *in vivo* on several lysine residues, namely, lysines 498, 505, 508, 521, and 524 (K498, K505, K508, K521, and K524, respectively), catalyzed by the acetyltransferase p300 (48). PARP-1 acetylation was stimulated *in vivo* by lipopolysaccharides and was required for interaction of PARP-1 with p50 and activation of NF-κB transcriptional activity. However, the relevance of this modification in the context of BER is currently unknown. Phosphorylation of PARP-1 *in vitro* by extracellular signal-regulated kinases 1 and 2 (ERK1/2) on serine 372 (S372) and threonine 373 (T373) has been demonstrated to be required for PARP-1 activity (49). Indeed, depletion of ERK1/2 by siRNA, but also mutation of S372/T373 within PARP-1, caused a reduction in PAR accumulation *in vivo* following alkylation-induced DNA damage. However, there is little recent evidence to support the idea of the importance of PARP-1 phosphorylation during cellular BER.

PARP-1 has been shown to be a target for SUMOylation. PARP-1 is SUMOylated both *in vitro* and *in vivo* by the SUMO E3 ligase PIASy using SUMO-1 and SUMO-2, which were localized to lysines 203 and 486 (K203 and K486) within the PARP-1 protein (50). PARP-1 SUMOylation using SUMO-2 was induced in cells in response to heat shock stress and was subsequently shown to be a target for RNF4-mediated ubiquitylation-dependent deg-

radation mediated by the E3 ubiquitin ligase ring finger protein 4 (RNF4). This was thought to act as a mechanism for clearance of PARP-1 but to do so specifically away from heat shock-inducible promoter regions. Additionally, PARP-1 has been shown to be SUMOylated in a PIASy-mediated manner on mitotic chromosomes and is modified by PIASy *in vitro* by SUMO-2 and SUMO-3 where lysine 482 (K482) is thought to be one of the primary sites of SUMOylation (51). Modification of PARP-1 by SUMO in this case did not cause an alteration in PARP-1 protein levels but instead was suggested to control poly(ADP-ribosyl)ation of chromatin-associated proteins. As mentioned above, PARP-1 acetylation by p300 was reported to be required for transactivation activity, and, intriguingly, cross talk between acetylation and SUMOylation of PARP-1 has been found (52). *In vitro* and *in vivo* SUMOylation of PARP-1 using either SUMO-1 or SUMO-3 was localized to lysine 486 (K486) on PARP-1 and prevented acetylation of the enzyme. SUMOylation also reduced coactivator activity and was therefore proposed to regulate gene expression, particularly expression of hypoxia-inducible genes. Finally, PARP-1 SUMOylation *in vitro* has been identified as being enhanced by intact undamaged DNA. *In vivo* SUMOylation of PARP-1 was discovered to occur predominantly on lysines 203 and 486 (K203 and K486, respectively) and was found to be associated with the protein binding to chromatin (53). This suggests a mechanism whereby the ability of PARP-1 to selectively bind intact DNA or damaged DNA defines whether its catalytic activity or its role as a coactivator regulated by SUMOylation is to be employed.

Modification of PARP-1 by ubiquitylation has been reported in a number of studies. PARP-1 was initially found to be polyubiquitylated in cells treated with a proteasomal inhibitor, an observation which was reproduced *in vitro* using rabbit reticulocytes as a source of enzymes catalyzing ubiquitylation (54). As mentioned above, the E3 ubiquitin ligase RNF4 has been shown to promote polyubiquitylation of the SUMOylated form of PARP-1 *in vivo* following overexpression of both proteins in cells (50). Another ring finger protein, Iduna/RNF146, has been demonstrated to ubiquitylate and cause degradation of PARP-1 *in vivo*, particularly following the induction of alkylated DNA damage. This enzyme was also able to target the poly(ADP-ribosyl)ated form of PARP-1 for ubiquitylation-dependent proteasomal degradation (55). Intriguingly, overexpression of Iduna caused increased resistance of cells to alkylation-induced stress, whereas siRNA-mediated knockdown of Iduna caused sensitization. However, *in vitro*, Iduna was shown to polyubiquitylate a number of DNA damage response proteins, including XRCC1 and Lig III (see section below); thus, the role of Iduna in this process is likely to be complex. Finally, the checkpoint with forkhead-associated and RING finger domain protein (CHFR) was identified as an E3 ubiquitin ligase able to polyubiquitylate PARP-1 both *in vitro* and *in vivo*, particularly in response to mitotic stress (56). This CHFR-induced polyubiquitylation was linked to proteasomal degradation of PARP-1, as *chfr* knockout cells contained elevated PARP-1 protein levels compared to the corresponding wild-type cells. Similarly to the observations with Iduna, CHFR was suggested to target the poly(ADP-ribosyl)ated form of PARP-1. This activated form of PARP-1 induced in response to mitotic stress showed a greater interaction with CHFR and thus was polyubiquitylated more readily. In a similar study, CHFR overexpressed in cells was demonstrated to be recruited to sites of laser microirradiation-induced DNA damage in a poly(ADP-ribosyl)ation-dependent manner. Poly(ADP-ribosyl)ated PARP-1 was also shown to be a target for CHFR-mediated polyubiquitylation (57). As with RNF4-mediated polyubiquitylation of SUMOylated PARP-1, this suggests a possible mechanism for displacing activated PARP-1 from DNA damage sites, therefore promoting DNA repair. Nevertheless, with the multiple proposed E3 ubiquitin ligases catalyzing the ubiquitylation of PARP-1, and the potential for cross talk with SUMOylation and acetylation on similar or adjacent lysine residues, further research is warranted to fully understand the importance of PTMs of PARP-1, particularly during BER of DNA damage.

APE1. Apurinic/apyrimidinic endonuclease 1 (APE1) represents the major AP endonuclease activity in human cells but can also act as a redox factor for transcription factors. Acetylation of APE1 at lysine 6 or 7 (K6 or K7, respectively) by p300 *in vitro* and *in vivo* was shown to promote binding to negative calcium response elements present in the parathyroid hormone gene and to regulate gene expression (58). APE1 acetylation *in vivo* was also shown to enhance binding with Y-box-binding protein 1 (YB-1) and promotes YB-1-mediated gene expression, including that of the multidrug resistance gene encoding MDR1 (59). Alternatively, APE1 can be acetylated *in vivo* at lysines 27, 31, 32, and 35 (K27, K31, K32, and K35, respectively) (60). It was demonstrated using an N-terminal deletion mutant that this region is important for efficient interaction of APE1 with RNA and nucleophosmin (NPM) *in vitro* and that this mutant displayed a cytoplasmic localization *in vivo*. Increased *in vitro* endonuclease activity of the deletion mutant was also observed. Therefore, it was predicted, rather than implicitly proven, that acetylation in this region may be important for modulating BER activity and RNA metabolism. Interestingly, there is a suggestion that APE1 acetylation may be deregulated in triple-negative breast cancer (61). A further role for APE1 acetylation at alternative sites within the protein is thought to be that of mediating protein-protein interactions. This is supported by evidence that deacetylation of APE1 by Sirtuin1 (SIRT1), both *in vitro* and *in vivo*, at K6 and K7 promotes interaction of the enzyme with XRCC1 (62) and thus stimulates BER. Since, as described above, acetylation of APE1 on K6 and K7 is thought to control redox activities, it would be interesting to understand how these events are potentially coordinated to promote both transcriptional and BER roles of APE1.

APE1 can be modified by phosphorylation, catalyzed by multiple different kinases, at several amino acid residues. However, the data are conflicting and unclear. Phosphorylation by the serine/threonine kinases casein kinase 1 (CK1) and PKC *in vitro* was shown not to impact the endonuclease activity of APE1, although phosphorylation by casein kinase 2 (CK2) was proposed to eliminate APE1 enzymatic activity (63). Conversely, a subsequent study demonstrated convincingly that phosphorylation of APE1 by CK2 *in vitro*, using either recombinant APE1 or nuclear extracts from APE1-transfected cells, does not alter APE1 endonuclease activity (64). Instead, this was proposed to stimulate redox capability for the transcription factor AP-1 and to increase binding to and thus transcription of AP-1-targeted genes. Redox activity of APE1 has also been shown to be stimulated by *in vitro* PKC-dependent phosphorylation and to occur following exposure of cells to DNA-damaging treatment (65). With regard to the inhibition of endonuclease activity of APE1 by phosphorylation, this was clearly demonstrated to be catalyzed by cyclin-dependent kinase 5 (Cdk5)/p35 *in vitro* at threonine 233 (T233) on APE1 (66).

Cdk5-dependent phosphorylation of APE1 was further observed in neurons *in vivo*, which caused an accumulation of DNA damage and contributed to neuronal death.

Interestingly, Cdk5-dependent phosphorylation, in addition to inhibiting endonuclease activity of APE1, has been proposed to enhance modification by another PTM, ubiquitylation. Indeed, a phosphomimetic mutant (T233E) of APE1 increased the enzyme's ubiquitylation-dependent degradation *in vivo* (67). This PTM, within the N-terminal domain of APE1, was catalyzed by mouse double minute homolog 2 (MDM2), but additional E3 ubiquitin ligases were implicated given that ubiquitylation was still evident using cells deficient in MDM2. Prior to that study, APE1 ubiquitylation within its N-terminal region by MDM2 had been reported *in vitro* and *in vivo*, in the absence of phosphorylation. Downregulation of cellular MDM2 was proposed to increase APE1 levels by preventing MDM2-mediated proteasomal degradation (68). However, by fractionation of human whole-cell extracts and using APE1 as a substrate in *in vitro* ubiquitylation assays, ubiquitin protein ligase E3 component N-recognin 3 (UBR3) was identified as the major E3 ubiquitin ligase acting on APE1 (69). This ubiquitylation of APE1 occurred on multiple lysine residues (K6, K7, K24, K25, K27, K31, K32, and K35) within the N-terminal region of the enzyme, and this led ultimately to proteasomal degradation. This was supported by the observation of significantly increased levels of APE1 in UBR3 knockout cells, and a consequent increase in genome instability. Since K6 and K7 have also been shown to be sites for PTM by acetylation, it would be interesting to examine a potential cross talk between ubiquitylation and acetylation in regulating APE1 stability and protein-protein interactions and its role as a redox factor.

PNKP. Polynucleotide kinase phosphatase (PNKP) acts as a 5′ kinase and a 3′ phosphatase at DNA strand break termini and therefore plays a role in strand break repair (SSB and double-strand breaks), as well as in BER. Two independent studies have shown that PNKP is amenable to phosphorylation *in vitro* and *in vivo* by ATM and DNA-dependent protein kinase (DNA-PK) on serines 114 and 126 (S114 and S126, respectively) of PNKP (70, 71). These phosphorylation events were shown to be required for PNKP activity or retention of PNKP at DNA damage sites but were particularly important for effective DNA double-strand break repair. However, phosphorylation of PNKP is also evident following oxidative stress, indicating that this PTM is important during BER of oxidative DNA damage (72). In fact, ATM-dependent phosphorylation was revealed to prevent *in vitro* and *in vivo* ubiquitylation-dependent degradation of PNKP on lysines 414, 417, and 484 (K414, K417, and K484, respectively), mediated by the Cul4A-DDB1-STRAP E3 ubiquitin ligase complex (72). Indeed, STRAP knockout cells contained elevated levels of PNKP due to deficient ubiquitylation activity and thus were more resistant to oxidative stress. ATM is therefore required to phosphorylate PNKP in response to oxidative DNA damage, inhibiting PNKP ubiquitylation and allowing the protein to accumulate in order to coordinate an efficient DNA damage response. Therefore, cross talk between phosphorylation and ubiquitylation plays a critical role in the regulation of PNKP. Interestingly, PNKP has recently been shown to interact *in vivo* with the deubiquitylation enzyme ataxin-3 (ATXN3), which enhances PNKP phosphatase activity (73). Therefore, it is possible that deubiquitylation of PNKP by ATXN3 is opposing Cul4A-DDB1-STRAP-mediated ubiquitylation of PNKP.

FEN-1. Flap endonuclease-1 (FEN-1) is a structure-specific endonuclease that plays a vital role in long-patch BER. FEN-1 was identified as a substrate for the p300 acetyltransferase *in vitro* (74) and was specifically modified on lysines 354, 355, 377, and 380 (K354, K355, K377, and K380, respectively). This FEN-1 acetylation was also discovered *in vivo*, being induced by treatment of cells with UV irradiation. Acetylated FEN-1 displayed reduced endonuclease activity in *in vitro* reconstituted systems containing oligonucleotide substrates with a 5′-flap structure and also exhibited reduced DNA binding, thereby acting as a potential mechanism for regulating enzymatic activity.

Phosphorylation of FEN-1 by the Cdk1-cyclin A complex was demonstrated both *in vitro* and *in vivo*, particularly during the end of the S phase of the cell cycle (75). A reduction in the flap endonuclease activity of phosphorylated FEN-1 was observed *in vitro*, even though DNA binding activity was not affected. Consequently, Cdk1-cyclin A was proposed to regulate FEN-1 by phosphorylation in a cell cycle-dependent manner. FEN-1 phosphorylation has also shown to be induced in response to UV irradiation, and this event caused translocation of the protein from the nucleoli into the surrounding nucleus to participate in DNA repair (76). Indeed, overexpression of a phosphomimetic mutant (aspartate) of FEN-1 at serine 187 (S187) caused this protein to be excluded from nucleoli. Interestingly, a cross talk between this phosphorylation event and two other protein modifications has been discovered by the same authors. First, FEN-1 was shown to be methylated *in vitro* by arginine methyltransferase 5 (PRMT5), predominantly on arginine 192 (R192), and this prevented phosphorylation at S187 (77). Methylated FEN-1 was induced in response to oxidative DNA damage, and extracts prepared from cells expressing a mutant form of FEN-1 that was unable to be methylated were defective in repair of flap-containing oligonucleotide substrates. This suggested that methylation of FEN-1 is required for efficient DNA damage repair. Second, phosphorylation of FEN-1 is thought to initiate a sequence of events leading to the degradation of the protein (78). Indeed, phosphorylation of FEN-1 at S187 *in vivo* was shown to promote SUMOylation using SUMO-3 on lysine 168 (K168). This subsequently enhanced the polyubiquitylation-dependent degradation of FEN-1, mediated by the E3 ubiquitin ligase pre-mRNA processing factor 19 (PRP19). Ultimately, this mechanism for the regulated control of FEN-1 protein levels, initiated during late S phase of the cell cycle, is thought to play a vital role in DNA replication rather than being related to the role of FEN-1 during long-patch BER. Nevertheless, this model requires further validation, particularly to understand the impact of these and other proposed PTMs of FEN-1 and their cross talk specifically during BER.

DNA POLYMERASES

Pol β. DNA polymerase β (Pol β) is the major DNA polymerase employed during BER, and several PTMs have been described that regulate both the activity and stability of the protein. It has been reported that Pol β can form a complex with the acetyltransferase p300, which acetylates the protein both *in vitro* and *in vivo* (79). The site of Pol β acetylation was identified as lysine 72 (K72), which is known to be the critical residue for Schiff-base formation during dRP lyase activity (80). Consequently, acetylated Pol β was less efficient *in vitro* in supporting reconstituted BER of oligonucleotide substrates containing site-specific DNA damage. Therefore, Pol β acetylation was suggested to act as a regulatory mech-

anism for BER by inactivating the enzyme when not required or by switching between short-patch BER and long-patch BER. Pol β has been shown to undergo methylation *in vitro* and *in vivo* by the activities of arginine methyltransferase 1 (PRMT1) and arginine methyltransferase 6 (PRMT6), with different cellular consequences. Methylation of Pol β predominantly on arginine 137 (R137) by PRMT1 caused no modulation in dRP lyase or DNA polymerase activities *in vitro* but appeared to inhibit the binding of the polymerase to PCNA (81). This methylation was predicted to prevent the involvement of Pol β in PCNA-dependent processes, including long-patch BER. In contrast, methylation of Pol β on arginines 83 and 152 (R83 and R152) by PRMT6 *in vitro* enhanced the binding of the enzyme to DNA and increased processivity (82). However, Pol β methylation did not affect reconstituted short-patch BER of DNA base damage-containing oligonucleotide substrates *in vitro*. Pol β modifications by acetylation and methylation have not been substantiated in more-recent studies, therefore bringing into question the importance of these PTMs in regulating Pol β and thus BER.

Pol β has been demonstrated, using an *in vitro* ubiquitylation system, to be polyubiquitylated within the 8-kDa N-terminal domain containing the β lyase activity by the E3 ubiquitin ligase CHIP (83). Cellular levels of Pol β were found to increase following siRNA knockdown of CHIP, whereas decreased protein levels of Pol β were observed following CHIP overexpression, demonstrating that CHIP modulates *in vivo* protein levels of Pol β. It was discovered that the stability of Pol β increases after formation of a repair complex with XRCC1-Lig III on damaged DNA, which prevents the protein from ubiquitylation-dependent degradation. Following that study, a second E3 ubiquitin ligase activity for Pol β, but this time catalyzing monoubiquitylation, was identified (84). Similarly to the CHIP results, monoubiquitylation within Pol β was found to occur within the 8-kDa N-terminal domain containing the β lyase activity by the E3 ubiquitin ligase Mule/ARF-BP1 *in vitro*. Ubiquitylation of Pol β was specifically localized to lysines 41, 61, and 81 (K41, K61, and K81, respectively). This monoubiquitylated form of Pol β was evident only in the cytoplasm of the cell and was a specific target for polyubiquitylation-dependent degradation catalyzed by CHIP. Consequently, an alteration in the protein levels of Pol β caused by siRNA-mediated downregulation of Mule, or by its inhibitory binding partner, the tumor suppressor protein ARF, was demonstrated to alter cellular DNA damage repair kinetics induced by oxidative stress. The hypothesis of ubiquitylation-dependent degradation of free Pol β, not involved in a repair complex, has also recently been supported. Interestingly, it was proposed that this was independent of CHIP and Mule and occurred on lysines 206 and 244 (K206 and K244, respectively) on the C-terminal end of the protein (85). However, that study employed a very artificial system using a particular cell line, specifically containing deletions in ARF, which was engineered for stable overexpression of Pol β rather than for monitoring endogenous protein as in the previous studies. Reversal of Mule- and CHIP-dependent Pol β ubiquitylation and, therefore, a complete mechanism of regulation of its steady-state levels were achieved by the identification of the deubiquitylation enzyme. This enzyme was identified as ubiquitin-specific protease 47 (USP47) and was able to regenerate the active, unmodified form of Pol β by removal of either single ubiquitin units or polyubiquitin chains attached to the cytoplasmic form of Pol β *in vitro* (86). siRNA-mediated knockdown of cellular USP47 was subsequently shown to cause a reduction in cytoplasmic Pol β protein levels, through Mule-dependent ubiquitylation, and therefore led to a delay in repair of oxidative and alkylated DNA base damage. USP47 depletion also elevated cell sensitivity to oxidative stress. Cumulatively, these studies demonstrated that cellular steady-state and DNA damage-induced protein levels of Pol β are finely controlled by ubiquitylation, through the UPP, and are responsive to the changing levels of endogenous DNA damage created through cellular oxidative metabolism.

Pol δ and ε. DNA polymerases δ and ε (Pol δ and ε) are DNA polymerases employed during long-patch BER. Only one publication to date has reported PTM of Pol δ, which involved modification of p68, one of the four subunits of the protein. Phosphorylation of p68 by protein kinase A (PKA) on serine 458 (S458) *in vitro* has been demonstrated (87). A phosphomimetic mutant (S458D) of p68, and of the Pol δ holoenzyme, was shown to display decreased binding to PCNA, and, consequently, decreased processivity of the polymerase was observed.

DNA LIGASES

Lig IIIα. DNA ligase IIIα (Lig IIIα) is known to be stabilized by forming a complex with the scaffold protein XRCC1, although XRCC1 itself is modulated by a number of PTMs. There are a number of reports clearly demonstrating that XRCC1 is phosphorylated by CK2. The first report demonstrated that XRCC1 was phosphorylated *in vitro* by CK2 on serine 518, threonine 519, and threonine 523 (S518, T519, and T523, respectively) and that this phosphorylation of XRCC1 was significantly reduced upon siRNA-mediated depletion of cellular CK2 *in vivo* (88). This CK2-dependent phosphorylation of XRCC1 did not change in response to alkylation-induced DNA damage and was not essential for cellular survival following this insult. It was discovered that phosphorylated XRCC1 binds *in vivo* to the forkhead-associated domain (FHA) of aprataxin, which was later identified as resolving abortive DNA ligation intermediates and is mutated in the neurological disorder ataxia oculomotor apraxia-1 (AOA1) (89). In a separate study, CK2 was found to phosphorylate XRCC1 similarly *in vitro* and *in vivo*, although this was found to include not only phosphorylation of S518, T519, and T523 but also phosphorylation spread across a minimum of eight sites within the C-terminal linker region of XRCC1 (90). Phosphorylated XRCC1 was shown to promote binding to a different FHA-containing protein, PNKP. This CK2-dependent process was demonstrated to be required for recruitment of both XRCC1 and PNKP to DNA damage repair foci and for the efficient repair of DNA single-strand breaks. Indeed, a mutant form of XRCC1 lacking these phosphorylation sites was defective in both these aspects. Furthermore, XRCC1 phosphorylation *in vivo* was shown to be required for effective dissociation of the protein from DNA, which then allows the protein to engage and promote efficient DNA damage repair (91, 92). This finding was further developed by demonstrating that CK2 present in the cytoplasm is indeed the major kinase catalyzing phosphorylation of XRCC1 on residues S518, T519, and T523 and that the majority of cellular XRCC1 is phosphorylated (93). In addition to promoting protein-protein interactions, that study demonstrated that phosphorylation of XRCC1 promoted protein stability by inhibiting ubiquitylation-dependent degradation. Therefore, depletion of CK2 by siRNA caused a reduction in the cellular levels of XRCC1 and consequently reduced DNA repair efficiency, highlighting a cross talk between phosphorylation and

ubiquitylation. Interestingly, XRCC1 has also been reported to be subject to phosphorylation by two other protein kinases, in addition to CK2. Checkpoint kinase 2 (Chk2) phosphorylates XRCC1 on threonine 284 (T284) *in vitro*, and this Chk2-dependent phosphorylation was induced in response to alkylation and oxidative-induced DNA damage *in vivo* (94). This was suggested to promote interaction of phosphorylated XRCC1 with the DNA glycosylases UNG and MPG and therefore potentially serves as a mechanism for repair protein recruitment and facilitating repair complex formation at DNA damage sites. Second, cellular XRCC1 was demonstrated to be phosphorylated by DNA-PK on serine 371 (S371) in response to IR-induced DNA damage. This was suggested to be required for the efficient repair of DNA double-strand breaks rather than DNA base damage and single-strand breaks (95). However, Chk2- and DNA-PK-mediated phosphorylation of XRCC1 and the requirement for cellular BER activity require further validation.

While modification of XRCC1 with SUMO *in vivo*, albeit with unknown function, has been reported (96), the protein has been shown to be susceptible to modification by ubiquitin, catalyzed particularly by the E3 ubiquitin ligase CHIP. Indeed, XRCC1 was demonstrated to be polyubiquitylated *in vitro* by CHIP, and an siRNA-mediated knockdown of CHIP caused an accumulation of cellular XRCC1 protein levels due to the lack of ubiquitylation-dependent degradation (83). The major site of *in vitro* and *in vivo* ubiquitylation of XRCC1 by CHIP was localized to the BRCA1 C terminus (BRCT II) motif on the C-terminal end of the protein, as a mutant form of XRCC1 lacking this domain was significantly more stable than the wild-type protein when expressed in cells. The same site of cellular XRCC1 ubiquitylation within the BRCT II motif was also confirmed in a separate study (92). CHIP has similarly been demonstrated to enhance the ubiquitylation-dependent degradation of XRCC1, but only when the protein was not bound to Pol β or heat shock protein 90 (HSP90) (85). In addition, XRCC1 has been shown to be ubiquitylated *in vitro* by the E3 ubiquitin ligase Iduna/RNF146, although this activity was dependent on modification of XRCC1 with PAR prior to ubiquitylation (55). However, a specific role for Iduna in modulating XRCC1 protein levels *in vivo*, was not investigated.

While XRCC1 has been demonstrated in several publications to be modified by phosphorylation and ubiquitylation, the DNA ligase that it forms a complex with and stabilizes, Lig IIIα, has been shown to be subject to PTMs in only a small number of reports. First, Lig IIIα was demonstrated to be phosphorylated by the protein kinase Cdk2 on serine 123 (S123) *in vitro*. This also occurred *in vivo* in a cell cycle-dependent manner, particularly in S and M phases (97). S phase-dependent phosphorylation of Lig IIIα was inhibited by cellular oxidative stress, and the mechanism involved inhibition of Cdk2 mediated by the protein kinase ATM. However, the importance of Cdk2-mediated phosphorylation for Lig IIIα function, and for BER, was not determined. Lig IIIα is also a reported target for ubiquitylation. As described for XRCC1 above, the E3 ubiquitin ligase Iduna/RNF146 can ubiquitylate Lig IIIα *in vitro* in a PAR-dependent manner (55). However, Iduna was demonstrated to interact with and ubiquitylate a number of PAR-modified DNA damage response proteins *in vitro*, including XRCC1 and Lig IIIα, for proteosomal degradation. Therefore, the full extent of Iduna's specific role in targeting cellular Lig IIIα (and XRCC1) for ubiquitylation remains to be revealed. Consequently, the importance of this enzyme, particularly during DNA damage repair operating through BER, is unclear. In contrast, Lig IIIα has been shown to be directly ubiquitylated *in vitro* by the E3 ubiquitin ligase CHIP and that CHIP controls the cellular levels of Lig IIIα, as revealed by increased protein levels following CHIP siRNA *in vivo* (83).

Lig I. DNA ligase I (Lig I) is the major DNA ligase employed in the final step of long-patch BER. However, to date, no characterized PTMs of this enzyme have been reported.

FUTURE PERSPECTIVES

It is clear that enzymes involved in the human BER pathway are specifically regulated by PTMs that mediate enzymatic activity, protein-protein interactions, cellular localization, or enzyme levels (summarized in Table 1). These PTMs, and the enzymes catalyzing them, therefore play key roles in coordinating efficient cellular DNA damage repair. This is particularly important in response to endogenous DNA damage created during oxidative metabolism and therefore contributes significantly to the maintenance of genome stability. Most of the reported data have focused on PTM of human enzymes involved in the postincision steps of BER and less so on the DNA glycosylases that are involved in identification and removal of damaged DNA bases. Since members of this class of enzymes initiate the BER pathway and excise potentially mutagenic DNA bases or those that block DNA transcription or replication, further research is needed to fully understand their mechanism of regulation by PTMs. However, there are additional data from proteomic screens (not covered in this review) which suggest that further PTMs of BER enzymes are present in the human genome. Therefore, the cellular consequences of these modifications and the enzymes catalyzing them have yet to be uncovered.

We have also highlighted any evidence for specific cross talk between different PTMs (e.g., phosphorylation activating/inhibiting ubiquitylation) which can effectively and efficiently modulate enzyme activity and levels, cellular localization, or DNA binding in order to modify the cellular DNA damage response accordingly. This is particularly important during the acute response to DNA damage to ensure that any fluctuation or accumulation in DNA damage, and thus any potential for increased mutations or aberrations, is quickly and effectively resolved. However, these regulatory "switches" in response to DNA damage must be accurately controlled since sustained, increased activities or levels of certain BER enzymes can lead to detrimental effects on genome integrity, the consequences of which are akin to those seen with enzyme deficiency. Nevertheless, the future challenge is to completely identify and characterize all the PTMs for each individual human BER protein and their potential for cross talk linked to a specific physiological role.

Intriguingly, some enzymes have been reported to target multiple BER proteins for PTMs, with either the same or different cellular consequences. For example, the acetyltransferase p300 can seemingly acetylate TDG, NEIL2, FEN-1, and Pol β and consequently reduce their activity and/or BER. In contrast, p300-mediated acetylation of OGG1, APE1, and PARP-1 can increase their enzymatic activities. In this instance, it is difficult to envisage that enzymes such as p300 would have such contrasting roles in controlling BER protein function, particularly during the cellular DNA damage response. This potential discoordination in the BER process could lead to the buildup of intermediates, including AP sites and DNA SSBs, that can promote mutations or formation of toxic double-strand breaks through stalled replication and/or transcription forks. Conversely, PKC has been reported to activate multiple BER proteins (MBD4, TDG, MYH, and APE1) by phos-

TABLE 1 Summary of the known characterized PTMs of human BER proteins and their functional consequences

Enzyme	Characteristic(s) (reference[s])[a]				
	Acetylation	Methylation	Phosphorylation	SUMOylation	Ubiquitylation
DNA glycosylases					
UNG2	N.D.	N.D.	↑ RPA binding (16); ↑ activity (17)	N.D.	↓ Protein levels (19–22)
SMUG1	N.D.	N.D.	N.D.	N.D.	↓ Protein levels (19–22)
MDB4	N.D.	N.D.	↑ Activity (23)	N.D.	N.D.
TDG	↓ APE1 binding (25)	N.D.	↓ Acetylation (26)	↓ DNA binding (27–30); ↓ acetylation (31)	↓ Protein levels (32, 33)
MYH	N.D.	N.D.	↑ Activity (35); ↓ DNA binding (36)	N.D.	↓ Protein levels (37)
OGG1	↑ Activity (38)	N.D.	↑ Activity (42)	N.D.	↓ Protein levels (43)
NTH1	N.D.	N.D.	N.D.	N.D.	N.D.
NEIL1	N.D.	N.D.	N.D.	N.D.	N.D.
NEIL2	↓ Activity (44)	N.D.	N.D.	N.D.	N.D.
NEIL3	N.D.	N.D.	N.D.	N.D.	N.D.
MPG	N.D.	N.D.	↑ Activity (46)	N.D.	N.D.
End processors					
PARP-1	↑ p50 binding (48)	N.D.	↑ PAR activity (49)	↑ Ubiquitylation (50); ↓ PAR activity (51); ↓ acetylation (52); ↑ DNA binding (53)	↓ Protein levels (50, 54–57)
APE1	↑ Gene regulation (58, 59); ↓ XRCC1 binding (62)	N.D.	↓ Activity (63, 66); ↑ redox activity (64, 65); ↑ ubiquitylation (67)	N.D.	↓ Protein levels (67–69)
PNKP	N.D.	N.D.	↑ Activity (70); ↑ DNA retention (71); ↓ ubiquitylation (72)	N.D.	↓ Protein levels (72)
FEN-1	↓ Activity (74)	↑ Activity (77); ↓ phosphorylation (77)	↓ Activity (75); ↓ nucleolar localization (76); ↑ SUMOylation (78)	↑ Ubiquitylation (78)	↓ Protein levels (78)
DNA polymerases					
Pol β	↓ dRP activity (79)	↑ Pol activity (82); ↓ PCNA binding (81)	N.D.	N.D.	↓ Protein levels (83–85)
Pol δ and ε	N.D.	N.D.	↓ PCNA binding (87)	N.D.	N.D.
DNA ligases					
XRCC1	N.D.	N.D.	↑ Aprataxin binding (88); ↑ PNKP binding (90); ↓ DNA binding (91, 92); ↓ ubiquitylation (93); ↑ MPG/UNG binding (94)	N.D.	↓ Protein levels (83, 85)
Lig III	N.D.	N.D.	N.D.	N.D.	↓ Protein levels (83)
Lig I	N.D.	N.D.	N.D.	N.D.	N.D.

[a] ↑, upregulation; ↓, downregulation; N.D., not determined.

phorylation, and furthermore, the E3 ubiquitin ligase CHIP has been shown to target OGG1, Pol β, XRCC1, and Lig IIIα for ubiquitylation-dependent degradation. In these cases, this would suggest that the activation of a single enzyme catalyzing PTMs can potentially impact BER at multiple levels (e.g., damaged base removal, DNA backbone incision, and gap filling). This would ensure that the activities and/or levels of BER proteins are effectively coordinated based on the levels of cellular DNA damage and that the efficiency of the BER process is accurately maintained, limiting the accumulation of BER intermediates. The difficulty in drawing these broad conclusions is that most of these findings are from individual, unsubstantiated reports which have been conducted in different models or cell lines. Ultimately, therefore, the global effect of such enzymes catalyzing PTMs of multiple BER proteins requires further validation, or, alternatively, the specific roles of these enzymes in BER modulation should be pinpointed.

Interestingly, the BER pathway is active in mitochondria; also, mitochondrion-specific forms of BER proteins, including UNG1 and seven isoforms of OGG1, exist in human cells. The mitochondrion is the major cellular source of reactive oxygen species, and mitochondrial DNA is therefore more susceptible to oxidative DNA damage than nuclear DNA. Consequently, an efficient BER

response is required to avoid damage and mutations in mitochondrial DNA, thus preventing genome instability. Despite this, we have little understanding of whether PTMs of BER proteins, characterized predominantly in the nucleus, are also occurring in the mitochondria. Therefore, a future focus should be on the identity and role of BER PTMs in this organelle and on understanding how these are impacted in the cellular DNA damage response.

It is also important to examine whether a defect in the molecular mechanisms regulating BER enzyme function, and thus DNA damage repair, through PTMs is associated with the development of human diseases. Indeed, there is increasing evidence that human BER protein levels are misregulated and, therefore, linked to premature aging and development of cancer and neurodegenerative diseases. However, the molecular mechanisms contributing to BER misregulation in these diseases remain largely unknown. Once this knowledge has been uncovered, it is clear that enzymes controlling BER PTMs may be novel targets for therapy using drugs or small-molecule inhibitors that, when combined with radiotherapy or chemotherapy (alkylating agents), may improve treatment and cure of specific human diseases.

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